

CLAIMS

- Sub 1
1. A cell composition consisting essentially of mammalian hematopoietic CXCR4⁺ stem and progenitor cells capable to migrate in response to stromal-derived factor 1 (SDF-1) and/or capable to adhere to stromal cells in response to an adhesion-inducing agent.
2. The cell composition according to claim 1, wherein the stem and progenitor cells are CD38^{-low} CXCR4⁺ cells.
- Sub 1
3. The cell composition according to claim 2, wherein the stem and progenitor cells are CD34⁺ CD38^{-low} CXCR4⁺ cells.
4. The cell composition according to claim 2, wherein the stem and progenitor cells are CD34⁻ CD38^{-low} CXCR4⁺ cells.
5. The cell composition according to ~~any one of claims 1 to 4~~, comprising also CD38^{high} stem and progenitor cells.
6. The cell composition according to claim 1, further comprising mammalian hematopoietic CXCR4^{-low} stem and progenitor cells that have the potential to express CXCR4 on the cell surface and are converted to CXCR4⁺ cells upon stimulation with a suitable agent.
7. The cell composition according to ~~any one of claims 1 to 6~~, wherein the stem and progenitor cells are autologous, allogeneic human cells from HLA-matched or HLA-nonmatched live donors or cadavers, or xenogeneic cells derived from a suitable non-human mammal.
8. The cell composition according to ~~any one of claims 1 to 7~~, wherein the stem and progenitor cells are human autologous cells.
9. The cell composition according to ~~any one of claims 1 to 7~~, wherein the stem and progenitor cells are human allogeneic cells.

9 10. The cell composition according to ~~any one of claims 1 to 9~~, wherein the cells are derived from bone marrow, cord blood, fetal liver, yolk sac or mobilized peripheral blood cells.

5 11. The cell composition according to claim 1, wherein said adhesion-inducing agent of CXCR4⁺ cells to stromal cells is selected from a cytokine, a lectin and a phorbol ester.

10 12. The cell composition according to claim 6, wherein said suitable agent capable of converting CXCR4^{-low} hematopoietic cells into CXCR4⁺ stem cells is selected from a lectin, a cytokine and/or stromal cells, said cytokines and stromal cells being those involved in maintenance, expansion and/or development of stem cells.

15 13. The cell composition according to claim 12, wherein said cytokine is selected from SCF, IL-1, IL-6, IL-11 and GM-CSF or a mixture thereof.

20 14. The cell composition according to claim 13, wherein said cytokine is SCF and said mixture of cytokines is a mixture of SCF and IL-6 or of SCF and GM-CSF.

25 15. A method for increasing the population of hematopoietic stem and progenitor cells for use in clinical transplantation, which comprises up-regulating surface CXCR4 expression of hematopoietic stem and progenitor cells and sorting out those CXCR4⁺ stem and progenitor cells that migrate in response to SDF-1.

30 16. The method according to claim 15, wherein said up-regulation is carried out by stimulation of a cellular population comprising hematopoietic CXCR4⁺ and CXCR4^{-low} stem and progenitor cells that have the potential to express CXCR4 on the cell surface, with a suitable agent, thus converting the CXCR4^{-low} into CXCR4⁺ cells, and sorting out those CXCR4⁺ stem and progenitor cells that migrate in response to SDF-1

17. A method for the preparation of a cell composition according to claim 1, comprising stimulating with a suitable agent a cell composition comprising hematopoietic CXCR4⁺ and CXCR4^{-low} stem and progenitor cells that have the potential to express CXCR4 on the cell

surface, thus converting the CXCR4^{-low} into CXCR4⁺ stem and progenitor cells, and sorting out those CXCR4⁺ stem and progenitor cells that migrate in response to SDF-1.

18. The method according to claim 16 ~~or 17~~, wherein said suitable agent is selected from a lectin, a cytokine and/or stromal cells and mixtures thereof, said cytokines and stromal cells being those involved in maintenance, expansion and/or development of stem cells.

19. The method according to claim 18, wherein said cytokine is selected from SCF, IL-1, IL-6, IL-11 and GM-CSF or a mixture thereof.

20. The method according to claim 19, wherein said cytokine is SCF and said mixture of cytokines is a mixture of SCF and IL-6 or of SCF and GM-CSF.

21. The method according to claim 18, wherein the CXCR4^{-low} cells that have the potential to express CXCR4 on the cell surface are stimulated with at least one type of mammalian stromal cells involved in maintenance, expansion and/or development of stem cells.

22. The method according to claim 21, wherein the stromal cells are mouse or human mesenchymal pre-adipocyte or osteoblast bone-forming stromal cells, or endothelial stromal cells.

23. The method according to claim 18, wherein stimulation is carried out with stromal cells and a cytokine or a mixture of cytokines.

24. The method according to claim 23, wherein stimulation is carried out with at least one type of stromal cells and SDF-1, SCF, IL-1, IL-6, IL-11 or GM-CSF or with a mixture of SCF and IL-6 or GM-CSF.

25. A method for increasing the population of hematopoietic stem and progenitor cells for use in clinical transplantation, which comprises inducing a cellular population of CXCR4⁺ stem cells to adhere to stromal cells in response to an adhesion-inducing agent and sorting out those CXCR4⁺ cells that adhered to the stromal cells in response to said agent.

26. The method according to claim 25, wherein said adhesion-inducing agent of CXCR4⁺ stem and progenitor cells to stromal cells is selected from a cytokine, a lectin and a phorbol ester.

5 27. The method according to claim 26, wherein said adhesion-inducing agent is SDF-1.

10 28. A chimeric non-human mammal transplanted with a human cell composition according to claim 1, said chimeric non-human mammal being capable of supporting the proliferation and differentiation of the transplanted immature human hematopoietic CXCR4⁺ stem and progenitor cells into all mature blood cells, including myeloid and/or lymphoid cells.

15 29. The chimeric non-human mammal according to claim 28, being a mouse transplanted with a cell composition consisting of human hematopoietic CD38^{-low} CXCR4⁺ stem and progenitor cells.

20 30. The chimeric non-human mammal according to claim 28, being a mouse transplanted with a cell composition consisting of human hematopoietic CD34⁺ CD38^{-low} CXCR4⁺ and/or CD34⁺ CD38^{-low} CXCR4⁺ stem and progenitor cells.

25 31. The chimeric non-human mammal according to claim ~~any one of claims 28 to 30~~, wherein the engraftment of the cells is carried out by a process comprising:

(a) sublethally irradiating an immunodeficient non-human mammal lacking a population of functional B and T cells; and

(b) transplanting into the irradiated immunodeficient non-human mammal the desired human hematopoietic CXCR4⁺ stem and progenitor cells.

30 32. The chimeric mammal according to ~~any one of claims 28 to 31~~, which is a NOD/SCID or a NOD/SCID β 2-microglobulin-knock out mouse.

33. An in vitro method for screening human immature hematopoietic CXCR4⁺ cells derived from bone marrow, cord blood, fetal liver, yolk sac or mobilized peripheral blood cells as candidates for transplantation into human hosts, said method comprising:

- 5 (a) measuring the level of cell surface CXCR4 expression in a separate sampling of cells with labeled anti-CXCR4 monoclonal antibodies;
- (b) increasing, if necessary, the level of CXCR4⁺ cells in the original sample by stimulation of CXCR4^{low} cells with a suitable agent;
- (c) measuring the CXCR4⁺ cells' ability to migrate in response to SDF-1 and/or to adhere to stromal cells in response to an adhesion-inducing agent; and
- (d) sorting out the CXCR4⁺ cells with a high migratory capability in response to SDF-1 and/or the cells which adhered to the stromal cells, these being the cells suitable for successful transplantation into human hosts.

10 34. An in vivo method for testing and ascertaining the engraftment capability of human hematopoietic cells derived from bone marrow, cord blood, fetal, yolk sac or mobilized peripheral blood cells, said CXCR4⁺ cells having a high migratory capability in response to SDF-1, or adhering to stromal cells in response to an adhesion-inducing agent, said method comprising:

- 15 (a) sublethally irradiating an immunodeficient non-human mammal lacking a population of functional B and T cells;
- (b) transplanting said human hematopoietic CXCR4⁺ cells into the irradiated immunodeficient mammal of (a); and
- 20 (c) measuring the level of mature human blood cells including myeloid and/or lymphoid cells in the obtained chimeric non-human mammal;

whereby stable engraftment in the model chimeric non-human mammal capable of supporting the proliferation and differentiation of said transplanted cells into all mature human blood cells, including myeloid and/or lymphoid cells, indicate the suitability of said cells for successful engraftment in human hosts.

25 35. A method for transplantation of immature hematopoietic cells in a patient in need therefor, said method comprising:

- 30 (i) conditioning the patient under sublethal, lethal or supralethal conditions; and
- (ii) transplanting the conditioned patient with a cell composition consisting essentially of human CXCR4⁺ stem and progenitor cells capable to migrate in response to SDF-1 and/or capable to adhere to stromal cells in response to an adhesion-inducing agent.

36. The method according to claim 35, wherein said human hematopoietic CXCR4⁺ stem and progenitor cells are autologous cells or from a HLA-matched or HLA-nonmatched live donor or cadaver.

5 37. The method according to claim 36, wherein said human immature hematopoietic CXCR4⁺ cells are derived from bone marrow, cord blood, fetal liver, yolk sac or mobilized peripheral blood cells.

10 38. The method according to ~~any one of claims 35 to 37~~, wherein said human cells are CD38^{-low} CXCR4⁺ cells.

39. The method according to claim 38, wherein said human cells are CD34⁺ CD38^{-low} CXCR4⁺ and/or CD34⁻ CD38^{-low} CXCR4⁺ cells.

15 40. The method according to claim 39, wherein the cell composition further comprises CD38^{high} cells.

20 41. The method according to ~~any one of claims 35 to 40~~, wherein said human CXCR4⁺ cells are obtained from bone marrow or by leukapheresis of peripheral blood from the donor after stimulation by a suitable cytokine.

42. The method according to ~~any one of claims 35 to 41~~, wherein the host patient is conditioned under sublethal conditions.

25 43. The method according to ~~any one of claims 35 to 41~~, wherein the host patient is conditioned under lethal or supralethal conditions

30 44. The method according to claim 43, wherein said lethal or supralethal conditions include total body irradiation (TBI), optionally followed by treatment with myeloablative and immunosuppressive agents.

45. The method according to claim 43, wherein said lethal or supralethal conditions include treatment with myeloablative and immunosuppressive agents without TBI.

46. The method according to ~~any one of claims 35-45~~, for the treatment of malignant diseases.

47. The method according to ~~any one of claims 35-46~~, wherein the transplanted cells are autologous cells.

48. A method for preparation of a cell composition consisting essentially of a cellular population of hematopoietic CXCR4⁺ pluripotent stem cells and committed progenitor cells capable to migrate in response to SDF-1, for autologous transplantation to a cancer patient, by ex vivo purging of malignant cells from a cancer patient while maintaining and enriching for normal hematopoietic CXCR4⁺ stem cells and progenitors, said method comprising:

- (i) providing hematopoietic stem and progenitor cells from a cancer patient, the malignant cells of which patient do not migrate to a chemotactic gradient of SDF-1;
- (ii) stimulating said hematopoietic stem and progenitor cells with a suitable agent to enhance their CXCR4 surface expression and response to SDF-1;
- (iii) carrying out an in vitro transmigration assay with the stimulated cells of (ii) to a gradient of SDF-1 across a mechanical barrier of cells in order to prevent spontaneous non-specific migration of malignant cells;
- (iv) washing the migrating cells to remove SDF-1; and
- (v) isolating the cells obtained in (iv),

said isolated cells being hematopoietic CXCR4⁺ stem and progenitor cells responsive to migration to SDF-1 and purged from the patient's malignant cells and suitable for autologous transplantation to the cancer patient.

49. The method according to claim 48, wherein the hematopoietic cells are derived from the patient's bone marrow or mobilized peripheral blood cells.

50. A method for the preparation of a cell composition consisting essentially of a cellular population of hematopoietic CXCR4⁺ pluripotent stem cells and committed progenitor cells capable to migrate in response to SDF-1, for autologous transplantation for the correction of genetic abnormalities, said method comprising:

- 5
- (i) introducing a normal gene in hematopoietic CXCR4⁺ stem and progenitor cells that migrate in response to SDF-1, from a patient having a genetic disorder;
- (ii) stimulating said transformed cells of (i) with a suitable agent to enhance their CXCR4 surface expression and response to SDF-1;
- (iii) carrying out an in vitro transmigration assay with the stimulated transformed cells of (ii) to a gradient of SDF-1 across a mechanical barrier of cells such as stromal cells or a stromal cell line;
- (iv) washing the migrating transformed cells to remove SDF-1; and
- 10 (v) isolating the transformed cells obtained in (iv),
said cells being hematopoietic CXCR4⁺ stem and progenitor cells responsive to SDF-1 containing the normal gene and being suitable for autologous transplantation to correct the patient's genetic disorder.

15 51. The method according to claim 50, wherein the hematopoietic cells are derived from the patient's bone marrow or mobilized peripheral blood cells.

52. A method of autologous transplantation of immature hematopoietic stem and progenitor cells for gene transfer to correct a patient's genetic disorder, which comprises:

- 20 (i) conditioning the patient under sublethal, lethal or supralethal conditions;
and
- (ii) transplanting the conditioned patient with a cell composition obtained according to the method of claim 50 or 51.

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